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GENETIC DIFFERENTIATION IN THE GENUS *PHIDIPPUS* (ARANEAE, SALTICIDAE)[1]

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ABSTRACT

Electromorph profiles of seven *Phidippus* species found in South Carolina were compiled by acrylamide gel electrophoresis. Twelve gene loci from 10 enzymes were analyzed. Mean heterozygosity of the seven species populations was 0.117 with an average proportion of polymorphic loci of 0.416. The mean genetic distance of all spider populations was 0.56 ± 0.04. *P. otiosus* (Hentz) and *P. regius* C. L. Koch were more closely related to each other (I = 0.781) than were any other pair of jumping spiders.

INTRODUCTION

Roach and Edwards (1984) reported the occurrence of seven species of *Phidippus* in South Carolina, and Terranova and Roach (1987) developed an electrophoretic key to identify these seven species in both immature and adult forms.

In a partial revision of the genus *Phidippus*, Edwards (1980) divided the genus into two subgenera. One subgenus contains the South Carolina species *Phidippus putnami* (Peckham and Peckham), and the other subgenus contains all other species observed in South Carolina.

According to the classification of Edwards (1980), *P. whitmani* Peckham and Peckham and *P. clarus* Keyserling belong to the *cardinalis* group. *P. audax* (Hentz), *P. otiosus* (Hentz) and *P. regius* C. L. Koch belong to the *audax* group, and *P. princeps* Peckham and Peckham belongs to the *princeps* group which Edwards (1980) states may be related to the *audax* group.

Edwards (1980) presented a cladogram of phylogenetic relationships among these seven species of *Phidippus* and others not reported from South Carolina based primarily on morphological characters. He further presented evidence of courtship relationships which supported his revised classification scheme (Edwards 1980).

In this report, we present information to elucidate the genetic relationships among the seven members of South Carolina *Phidippus*. This information is

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Table 1.—Enzyme systems studied and their polymorphic (P) or monomorphic (M) status in one or all species of *Phidippus*. Presumed subunit structure of observed electromorphs is indicated.

Enzyme	Enzyme commission number	Abbreviation and locus	Polymorphic (P) or monomorphic (M)	Subunit[a] no.
Aspartate aminotransferase	2.6.1.1	Aat-1	P	2
Amylase	3.2.1.1	Amy-1	P	1
Fumarase	4.2.1.2	Fum-1	M?	?
α-Glycerophosphate dehydrogenase	1.1.1.8	α-Gpd-1	P	?
Isocitrate dehydrogenase	1.1.1.42	Idh-1	P	2
		Idh-2	P	2
Malate dehydrogenase	1.1.1.37	Mdh-1	P	2
		Mdh-2	P	2
Phosphoglucose isomerase	5.3.1.9	Pgi-1	P	2
Phosphoglucomutase	2.7.5.1	Pgm-1	P	1
Superoxide dismutase	1.15.1.1	Sod-1	M	?
Tyrosinase	1.10.3.1	Tyr-1	P	1

[a]1 = Monomeric protein; 2 = dimeric proteins.

derived from the patterns of genetic variability at 12 presumptive gene loci in each *Phidippus*. The techniques of polyacrylamide gel electrophoresis and isozyme detection were used to obtain this information.

METHODS

Spiders used in these studies were either field-collected *Phidippus* mainly from eastern South Carolina or progeny obtained from laboratory-reared spiders. The species *P. audax*, *P. clarus*, and *P. princeps* occurred widely and were fairly abundant, whereas *P. otiosus*, *P. putnami*, *P. regius*, and *P. whitmani* were rarely collected due to their scarcity or their habitat specificity.

The electrophoretic techniques and the isozyme staining techniques used have been previously described (Terranova 1978, 1980, 1981a,b). A total of 12 gene loci represented by 10 enzyme systems were studied in every species in which they were present (Table 1).

Analysis of electrophoretic data was accomplished using program GELDAT (Terranova and Smith unpub.). Population statistics and dendograms were based on Ferguson (1980). Genetic divergence between species was determined by the genetic similarity (I) and genetic distance (D) statistics of Nei (1972). Similarity values (I) are a measure of the proportion of loci at which the allelic constituents of two populations are essentially identical. Similarity values range from zero (no identity) to one (complete identity). Genetic distance (D) values estimate the effective number of complete allelic substitutions per locus which have accumulated since any two populations diverged from a common ancestor.

RESULTS

The data presented in Table 2 show that the percent of polymorphic loci ranges from 16.7% in *P. putnami* to 58.3% in *P. audax*, *P. clarus*, and *P. whitmani* with

Table 2.—Levels of genetic variation in seven *Phidippus* species observed in South Carolina.

Species	Individuals sampled	Mean % ± S.E. heterozygotes expected per locus	Percent polymorphic loci[a]	Mean no. alleles per locus
P. audax	58	17.2 ± 5.0	58.3	1.83
P. clarus	38	11.8 ± 4.3	58.3	1.83
P. otiosus	11	6.1 ± 3.9	25.0	1.33
P. princeps	33	17.3 ± 5.7	50.0	1.75
P. putnami	7	5.9 ± 4.3	16.7	1.17
P. regius	21	4.7 ± 3.3	25.0	1.25
P. whitmani	10	17.9 ± 5.1	58.3	1.75

[a]Frequency of the most common allele ≤0.99.

an average for all seven species of 41.6% out of 12 loci. The expected mean frequency of heterozygous individuals per locus ranges from 4.7% in *P. regius* to 17.9% in *P. whitmani*, and the average for the genus is 11.7 ± 4.5%. The lower percent of heterozygotes per locus observed in the species *P. otiosus*, *P. putnami*, and *P. regius* as compared to the remaining species is probably due to the small number of field-collected spiders. For example, the low heterozygosity observed in *P. regius* is more than likely the result of most specimens belonging to the same family. In this case, only one wild adult was ever found. The remaining specimens were collected as spiderlings, presumably from the same mother. The electromorph [= allele, see Berlocher (1980) for discussion] frequencies at the 12 genetic loci as shown in Table 3.

The levels of genetic variation found in the genus *Phidippus* compared to other invertebrates show that they are characteristic of invertebrates in general. The data presented by Ferguson (1980) show a mean percent polymorphism of 51% and a mean percent heterozygosity of 15.5% for a wide assortment of invertebrates compared to 41% and 11.7%, respectively, for *Phidippus*.

Estimates of genetic similarity (I) and genetic distance (D) between all species pairs are given in Table 4. Genetic distance ranges from 0.247 for *P. regius* vs. *P. otiosus* to 0.846 for *P. princeps* vs. *P. putnami* with an average value of D for all pair-wise comparisons of 0.56 ± 0.04. Thus, on average, about 0.56 electrophoretically detectable electromorphic substitutions per locus have occurred in the separate evolutions of any two species.

Figure 1 shows the distribution of genetic similarities among loci. Generally, pairs of species are essentially identical (I = ≤0.95) at nearly 52% of the loci and completely different (I = ≤0.005) at about 32% of the loci, and few loci have genetic similarities in the broad range between 0.05 and 0.95. Again, our data correlate nicely with those reported for other invertebrate species (e.g. *Drosophila willistoni* group; Ayala et al. 1974a,b). Ferguson (1980) comments that this U-shaped distribution may be representative of most outcrossing sexual organisms. Among others, Ferguson (1980) and Avise and Ayala (1976) state that the significance of this bimodality of distribution is that for a given locus, any two species are either identical in electromorphic composition or completely distinct with unique electromorphs. This is important from a systematist's point of view in that it is not essential to sample many individuals at each locus since electromorphic frequency differences are not an important feature of interspecific studies (Ferguson 1980).

Table 3.—Frequencies of electromorphs in seven species of *Phidippus*.

Gene &	electro-	Species						
	morph	*audax*	*clarus*	*otiosus*	*princeps*	*putnami*	*regius*	*whitmani*
Aat-1	36	-	0.013	-	-	-	-	-
	46	0.053	-	-	-	-	-	-
	55	0.947	0.987	1.000	1.000	1.000	1.000	1.000
Amy-1	23	-	0.048	-	-	-	-	-
	30	-	0.077	-	-	-	-	-
	34	-	-	0.667	-	-	-	-
	36	0.121	-	-	-	-	-	-
	40	-	0.712	0.208	0.204	-	0.976	0.182
	45	0.638	0.067	0.125	0.278	1.000	0.024	0.500
	50	0.233	-	-	0.315	-	-	0.273
	55	-	0.096	-	0.093	-	-	-
	58	0.009	-	-	-	-	-	-
	63	-	-	-	0.111	-	-	0.045
Fum-1	55	-	1.000	1.000	1.000	-	-	-
	58	1.000	-	-	-	1.000	1.000	1.000
Idh-1	38	0.588	0.900	-	0.222	-	-	0.786
	50	0.412	0.100	1.000	0.778	1.000	1.000	0.214
Idh-2	55	0.921	1.000	1.000	0.852	1.000	1.000	0.938
	65	0.079	-	-	0.148	-	-	0.063
α-Gpd-1	116	-	-	-	0.256	-	-	0.167
	120	1.000	-	-	-	-	-	-
	124	-	1.000	1.000	0.750	-	1.000	0.917
	129	-	-	-	-	1.000	-	0.083
Mdh-1	21	-	0.056	0.062	-	-	-	-
	25	0.589	-	-	-	0.429	-	-
	30	-	0.944	-	-	0.571	-	-
	35	0.411	-	0.938	1.000	-	0.700	1.000
	43	-	-	-	-	-	0.300	-
Mdh-2	123	0.905	0.941	0.938	0.950	0.786	0.900	0.836
	143	0.095	0.059	0.062	0.050	0.214	0.100	0.164
Pgm-1	65	-	-	-	1.000	-	-	0.600
	70	1.000	0.067	1.000	-	1.000	1.000	0.400
	76	-	0.333	-	-	-	-	-
Pgi-1	45	-	0.041	-	0.147	-	-	-
	55	1.000	0.959	1.000	0.853	1.000	1.000	-
	60	-	-	-	-	-	-	0.143
Sod-1	134	-	-	1.000	-	-	-	-
	138	-	-	-	-	-	1.000	-
	143	-	-	-	-	1.000	-	-
	156	-	1.000	-	1.000	-	-	-
	159	1.000	-	-	-	-	-	1.000
Tyr-1	27	0.066	-	-	-	-	-	-
	33	0.920	1.000	-	-	-	-	-
	37	-	-	-	-	1.000	-	-
	44	0.020	-	1.000	1.000	-	1.000	1.000

In order to more easily visualize the information in Table 4, we evaluated the data by an agglomerative clustering procedure, the unweighted pair-group method with arithmetic means (UPGMA, Sneath and Sokal 1973). The resultant dendogram is presented in Figure 2. The relationship based on the biochemical similarities between *Phidippus* species are discussed in relation to the interpretations of Edwards (1980).

Table 4.—Matrix of genetic distance (above diagonal) and genetic similarities (below diagonal) between species in *Phidippus* based on 12 loci. Distances and similarities calculated using the method of Nei (1972).

	Species						
Species	*audax*	*clarus*	*otiosus*	*princeps*	*putnami*	*regius*	*whitmani*
P. audax	-	0.558	0.633	0.771	0.423	0.672	0.443
P. clarus	0.573	-	0.480	0.416	0.763	0.726	0.752
P. otiosus	0.531	0.619	-	0.261	0.647	0.247	0.502
P. princeps	0.463	0.660	0.770	-	0.846	0.416	0.402
P. putnami	0.655	0.466	0.524	0.429	-	0.672	0.829
P. regius	0.511	0.484	0.781	0.659	0.511	-	0.301
P. whitmani	0.642	0.471	0.606	0.669	0.437	0.740	-

DISCUSSION

The cladogram presented by Edwards (1980) depicting hypothetical phylogenies of the *Phidippus* species was constructed utilizing morphological characters considered primitive or derived. His observations on the overall courtship behaviors of *Phidippus* proved to be a good indicator of these phylogenetic relationships (Edwards 1980).

Both the cladogram of Edwards, based on morphological characters, and our dendogram based on biochemical electromorph differences agree in one important aspect; that of the relationship between *P. otiosus* and *P. regius*.

Edwards (1980) stated that the two species, *P. otiosus* and *P. regius*, appeared to be closely related, and further, that *P. otiosus* is assumed to be the parent species because it is hypothesized that *P. regius* evolved as an isolated population on islands formed when the Florida peninsula was partially submerged during

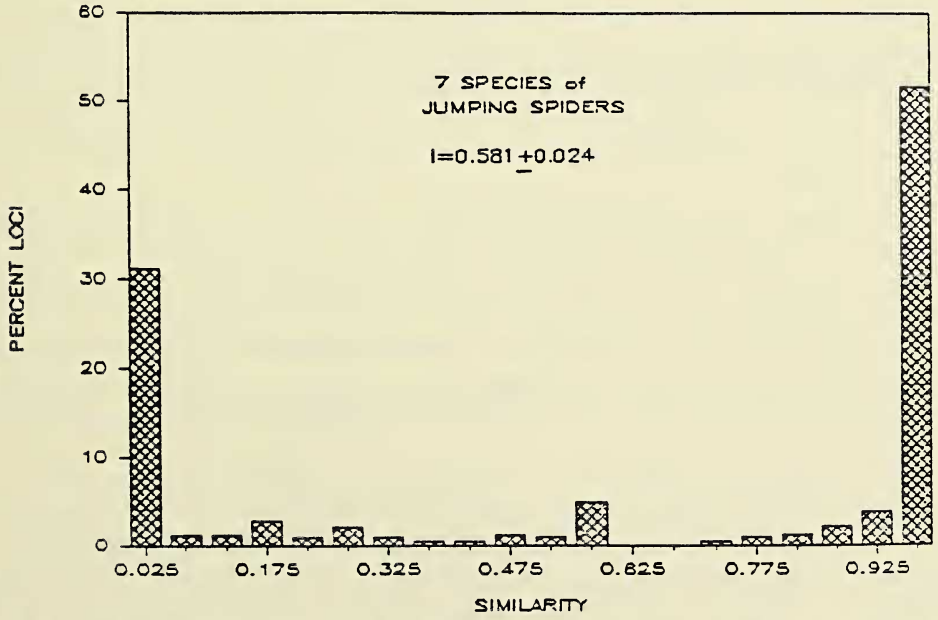

Fig. 1.—Percentage of loci within a given range of genetic similarity values in the comparisons among seven species of South Carolina *Phidippus*.

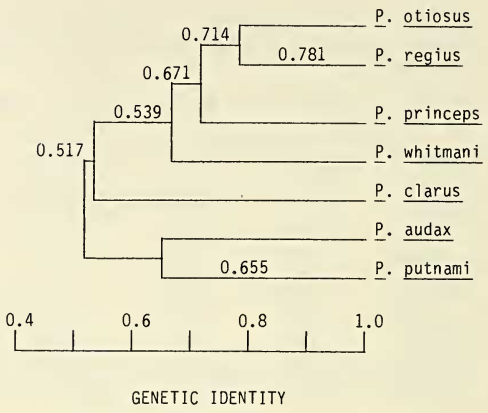

Fig. 2.—Dendogram of South Carolina *Phidippus* based on the unweighted pair group method with arithmetic mean (UPGMA). Scale is in units of genetic identity, I.

prehistoric times. He based these assumptions on morphological similarities, and on field and laboratory observations. Morphologically, the basic structure of body markings and the genitalia are similar in both species, but the color patterns are different. The ranges of both species overlap greatly, and both species have been observed sharing the same ecological niche. The most convincing evidence presented by Edwards (1980), however, is the fact that hybridization attempts between *P. otiosus* males × *P. regius* females were successful in the laboratory and produced viable young. Edwards (1980) also collected apparent hybrids in xeric woods habitat, and he recorded that a colleague, Dr. John F. Anderson, collected a male *P. otiosus* in copula with a female *P. regius*, 19 September 1980 (Edwards 1980).

Our results support Edwards' hypothesis that these two species are closely related. From the paired comparisons of Table 4, these two species show the least overall genetic divergence (D = 0.247) and the most overall genetic identity (I = 0.781). Thus, about 24.7 electrophoretically detectable electromorphic substitutes per 100 loci have occurred between *P. otiosus* and *P. regius* as compared to 84.6 between *P. princeps* and *P. putnami* or compared to an average of 56 substitutions per 100 loci in the evolution of the genus as a whole. These results compare favorably with other surveys in which hybridizing species are completely distinct in allelic composition at almost one third to one half of their loci (Avise 1974).

These results are interesting when looked at in light of Edwards' (1980) discussion on hybridization in which he states that hybridization was successful between *P. otiosus* and *P. regius* from a similar part of their range (northern peninsular Florida), but unsuccessful when attempts to hybridize the same two species were made with individuals from widely separated sources. A thorough study of these two species throughout their ranges by isozyme analysis and hybridization studies could reveal a number of significant factors concerning their evolution.

Other similarities between the cladogram of Edwards (1980) and our dendrogram exist. Edwards states that the courtship behavior of *P. clarus* has more in common with the *audax* group than to its apparent morphological relatives in the *cardinalis* group. The relationship of *P. clarus* to *P. otiosus* and *P. regius* in our data would tend to substantiate this in that the latter two species are classified by Edwards as belonging to the *audax* group. Our data, like Edwards', also show that *P. clarus* and *P. whitmani* are related. However, our

data differ from Edwards' in that *P. audax* is more distantly related to the previously named species. *P. princeps* is apparently more related to the *P. witmani*, *P. otiosus*, *P. regius* group than to *P. audax*. This would concur with Edwards' premise that *P. princeps* may be related to the *audax* group. Our data also differ from Edwards' in that *P. putnami* is also distantly related to *P. audax*.

Overall, our results fit the classification of Edwards with the exception of *P. audax*. By including other congeneric species, a more certain phylogeny is possible for the genus *Phidippus*, as has been shown with other invertebrate groups. In *Drosophila* for example, Nair et al. (1971), Yang et al. (1972), Lakovaara et al. (1972) found agreement between similarity based on classical systematic criteria, and those based on electrophoretic data. Avise (1974) concluded that attempts to utilize electrophoretic data for classifying closely related species would appear justified.

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